Draft Discovery Bay Quality Assurance Project Plan

Version 1.0

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Acronyms and Abbreviations

ASTM	American Society for Testing and Materials
CLP	Contract Laboratory Program
DQO	Data Quality Objective
DMP	Data Management Plan
EDD	electronic data deliverable
EPA	U.S. Environmental Protection Agency
ESAT	Environmental Services Assistance Team
ESU	Environmental Services Unit
FASTAC	Field and Analytical Services Teaming Advisory Committee
FD	field duplicate
FP	Field Procedures
FTL	Field Team Leader
GPS	global positioning system
HSP	Health and Safety Plan
IAG	Interagency Agreements
MDL	Method Detection Limit
MEL	EPA R10 Manchester Environmental Laboratory
MRL	Method Reporting Limit
MS	matrix spike
MSD	matrix spike duplicate
PBDE	polybrominated diphenyl ethers
PCB	polychlorinated biphenyl
PID	photoionization detector
PM	Project Manager
PO	Project Officer
QA	Quality Assurance
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
QC	Quality Control
R10	EPA Region 10

RAS	Routine Analytical Services
RI	Remedial Investigation
RL	Reporting Limit (synonymous with Method Reporting Limit, MRL)
RPD	Relative Percent Difference
RSCC	Regional Sample Control Coordinator
RSD	relative standard deviation
RTL	Review Team Leader
SMO	Sample Management Office
SOP	Standard Operating Procedure
SRM	standard reference material
SU	sampling unit
SVOC	semi-volatile organic compound
TAL	target analyte list
TEQ	toxic equivalence
TOC	total organic carbon
TOPO	Task Order Project Officer
VOC	volatile organic compound

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SECTION 1

Introduction

This Quality Assurance Project Plan (QAPP) presents the policies, organizations, objectives, and functional activities/procedures for the Discovery Bay and Port Discovery Seafarms Sampling Tasks being conducted by the U.S. Environmental Protection Agency (EPA) at Port Discovery Seafarms and vicinity in Discovery Bay, Jefferson County, Washington. The QAPP and its supporting documents, found in Appendix A (Systematic Planning/Data Quality Objectives [DQOs] for Event 1), Appendix B (Field Procedures [FP] for Event 1), Appendix C (Site-specific Data Management Plan) and Appendix D (Health and Safety Plan [HSP]) have been developed to document the type and quality of data needed for environmental decisions.

This investigation will focus on identifying if conditions exist at the time of sampling that cause oyster larval mortality similar to that experienced at Port Discovery Seafarms in 2015. If a similar level of mortality is observed in the Discovery Bay waters collected in this study that is not observed in the control population, EPA or others may conduct additional work aimed at identifying potential causes. If oyster larval mortality is not observed, additional work will be limited.

This QAPP evaluates information that can be collected and evaluated by EPA. The mortality event that led to this study occurred during July and August of 2015. The sampling below will be conducted in 2017.

1. Chemical analysis of oyster shells from Port Discovery Seafarms and Discovery Bay (reference area), to identify if metal concentrations in the shells differ between the two locations. This data may then be used to support a statement about the cause of the 2015 observed mortality and/or discoloration and deformation of shells in adult shellfish (Table 2.1, Activity 4)
2. Determination of whether Discovery Bay water causes significantly more mortality in larval oysters than water from reference and control locations (Table 2.1, Activity 1). If so, toxicity testing of water from these locations may occur, including adjusting water to different pH and ammonia levels, to determine if chemical changes associated with ocean acidification and/or nutrient status are associated with oyster mortality (Table 2.1, Activities 2 and 3)

These tasks will be followed by a data evaluation period to determine if the DQOs for the study have been achieved or if additional or different data are needed. One or more additional QAPP addendums may be developed to fill data gaps identified by evaluation of this data. Additional EPA involvement may be affected by the agency's ability to dedicate resources to the effort.

This QAPP follows EPA guidelines contained in *EPA Guidance for Quality Assurance Project Plans* (EPA, 2002a), and *EPA Requirements for Quality Assurance Project Plans* (EPA, 2001, reissued 2006).. The development, review, approval, and implementation of the QAPP is part of EPA's mandatory quality system, which requires all organizations to develop and operate management structures and processes in order to ensure that data used in agency decisions are of the type and quality needed for their intended use.

EPA conducted a thorough evaluation of the literature and spoke with NOAA, EPA and other experts regarding the availability of methods to support this project. The data/method need outlined in the first two steps of this work cannot be obtained using published EPA or ASTM methods, and requires the development of new methods. These research methods will be developed specifically to meet the data quality objectives of this project. Methods Activities 1 and 2 (in Table 2.1) are within the capability of the EPA's staff and are being developed at our Region 10 Laboratory. Both of these studies will be conducted during the first phase of this project. Activities 3 and 4, have not yet been initiated, and will only be considered if the oyster mortality observed in 2015 is reproduced. Because method development for these activities has not yet been initiated, the feasibility to conduct these methods has not been confirmed.

This document is organized as follows:

- **Section 1—Introduction.** Provides the purpose and organization of this report.
- **Section 2—Project Management.** Provides a summary-level description of the project and task organization; background and problem definition; work tasks and project schedule; quality and objectives criteria; special training and certifications; and documents and records.
- **Section 3—Data Generation and Acquisition.** Describes the sampling design; sampling methods; sample handling and custody; analytical methods; quality control; instrument, equipment testing, inspection and maintenance; instrument/equipment calibration and frequency, inspection/acceptance of supplies and consumables; nondirect measurements; and data management.
- **Section 4—Assessment and Oversight.** Describes assessment, oversight, and reports to management.
- **Section 5—Data Validation and Usability.** Introduces the concepts of data review, verification, and validation; describes verification and validation methods; and explains reconciliation with user requirements. This section also presents the test acceptability criteria for the water column toxicity tests that will be performed during this work.
- **Section 6—References.** Provides a list of references used in this document.

In addition to the sections summarized above, this QAPP contains the following appended materials:

- Appendix A—Systematic Planning/Data Quality Objectives
- Appendix B – Field Procedures for Event 1
- Appendix C – Site Specific Data Management Plan
- Appendix D – Health and Safety Plan
- Appendix E – Larval-Juvenile Bivalve Toxicity Test for Pacific Oyster (*Crassostrea gigas*): Step-by Step Summary

Project Management (EPA Group A)

2.1 Project/Task Organization (A4)

The Project Manager (PM) manages the financial, scheduling, and technical aspects of the work. The key people involved in interfacing with the PM are the Quality Assurance Officer (QAO), Task Leader, and Field Team Leader (FTL).

The data flow is summarized on Figure 2-1. The data for this task order are limited to laboratory analyses and sample records.

The following additional organizational guidelines apply:

- The review team (led by the QAO) will review project planning documents, data evaluation, and deliverables. The primary responsibility for project quality rests with the PM, and independent quality control is provided by the QAO, Project Chemist and Task Leader.

Where quality assurance problems or deficiencies requiring special action are uncovered, the PM and QAO will identify the appropriate corrective action to be initiated by the .

Project Manager – Linda Anderson-Carnahan, Margo Young:

- Manages the financial, scheduling, and technical aspects of the work.
- Communicates with organizations/parties associated with this project but external to EPA.

Regional Quality Assurance Manager – Donald Brown, or designee:

- Reviews and approves the QAPP and any associated project documentation.
- May conduct assessments of field activities.

Regional sample Control Coordinator (RSCC) – Jennifer Crawford/Don Matheny(alternate):

- Reviews QAPP and provides guidance for sample management, field sampling, and data management/Scribe.
- Coordinates and communicates requirements associated with QA and sample control.
- Coordinates and schedules sample analyses performed through EPA Manchester Environmental Lab (MEL)
- Assigns unique sample identification numbers along with Region 10 project codes for tracking.
- Provides issue resolution for R10 analyzed samples between the lab and project field/sampling staff.
- EPA Scribe/data management point of contact; reviews all R10 Scribe Deliverables for adherence to the requirements in this QAPP and in the EPA Region 10 Data Management Plan for Environmental Monitoring and Associated Geospatial Data (EPA 2014).

Quality Assurance Chemist – Donald Brown:

- Reviews and approves the QAPP and any associated project documentation as an alternate to the RQAM.
- Provides data validation, as necessary, for laboratory analyses.

MEL Supervisory Chemist – Gerald Dodo:

- Main contact between MEL and project personnel.
- Coordinates with lab team leaders on sample analysis, data review, and reporting.
- Oversees laboratory responsibility to conduct analyses in accordance with their QA Manual, the NELAC Institute (TNI) Accreditation requirements, and the criteria in this QAPP.
- Authorizes acceptance of samples into MEL and the release of final reviewed data.

EPA MEL Technical Lead – Katie Adams:

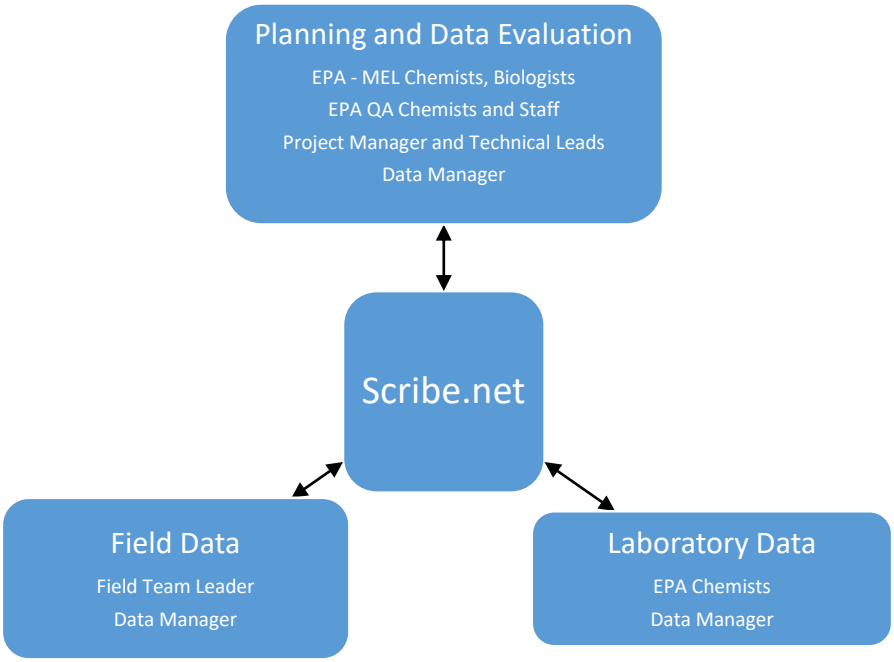
- Oversees R10 inorganic laboratory analysis and preparation of laboratory final data and reports in accordance with the QAPP requirements and analytical methodology specified for the project along with laboratory SOPs.
- Coordinates and conducts method development activities as described in this QAPP.

Project Toxicologist – Burt Shephard:

- Provides guidance on all aspects of the project, from sampling scheme through data analysis.
- Assists in methodology consultation, data analysis, and writing of the final report.

Field Team Leader – TBD

FIGURE 2-1
Data Flow Summary



- A QAPP and R10 Analytical Services Request Form are required for the RSCC to begin laboratory coordination. The R10 laboratory is offered first right of refusal before proceeding to Tier 2. For the oyster shell analyses and initial set of toxicity tests, it is anticipated that all work will be done at MEL. RSCC lab coordination occurs after QAPP development. Therefore, laboratory and analytical specifics throughout the QAPP must be applicable to the EPA R10 Manchester Environmental Laboratory (MEL). Laboratories are required to meet the analytical requirements set forth in this QAPP for methodology, method reporting limits (MRLs), quality control, and data management. The laboratory data flow is presented in Figure 2-2.
- The EPA RSCC is responsible for EPA R10 and MEL coordination. The RSCC works with the EPA labs and the project's PMs in resolving laboratory and field quality assurance (QA) issues and laboratory scheduling. The RSCC provides the regional sample tracking numbers, Scribe training, custody seals, and other required chain-of-custody documentation.

2.2 Problem Definition/Background (A5)

2.2.1 Background

Port Discovery Seafarms, a Pacific oyster (*Crassostrea gigas*) and Manila clam (*Venerupis philippinarum*, formerly *Ruditapes philippinarum*) floating aquaculture facility located on the southeastern shoreline and adjacent waters of Discovery Bay, Jefferson County, Washington, suffered mortality events in July and August of 2015. The owner reported a visible plume in Discovery Bay surface water during this time that coated the beach and equipment at the facility. The following adverse effects were among those observed on shellfish at the time of the observed plume, according to a February 16, 2016 shellfish pathology report prepared by the firm AquaTechnics of Carlsborg, WA:

- 1.5 mm size oyster seed stopped growing
- Shells of exposed stock turned black

- Oysters had developed a white deposit along the edge of their valves
- 90% of oyster seed of a size less than 75 mm died
- 20% of market sized oysters died
- Surviving seed oysters had abnormal shells – both valves grew in concave downward direction
- A ridge of white material formed inside the margin of the valves of the surviving oysters
- Market sized manila clams became weak and suffered substantial mortality
- No Manila clam seed was on the site during July and August 2015
- Mussels on the site suffered 90% mortality

The owner of Port Discovery Seafarms, Tom Madsen, through his representative Congressman Derek Kilmer, referred the Site to EPA due to concerns about hazardous substances possibly leaching from nearby salmonid habitat remediation activities to Discovery Bay surface water, with subsequent impacts on development and survival of shellfish at Port Discovery Seafarms. Several other possible causes of the mortality events, including release of algal toxins from blooms known to occur in Discovery Bay, and ocean acidification, cannot be ruled out at this time.

Characterization of areas near Port Discovery Seafarms and other locations within the adjacent portions of Discovery Bay is needed to identify the cause(s) of the 2015 shellfish mortality events.

The study area for the Discovery Bay and Port Discovery Seafarms Site is shown in Figure 2-3. Based on Washington Department of Health locations for permitted shellfish growers, the approximate surface water sampling locations are as follows:

Port Discovery Seafarms: 48°.00585 -122°.83902

Snow Creek Oysters: 48°.08175 -122°.89039

The actual sampling locations will be determined and recorded in the field based on the actual location of the Port Discovery Seafarms water intake structure, and a location at Snow Creek that will not interfere with the submerged oyster racks.

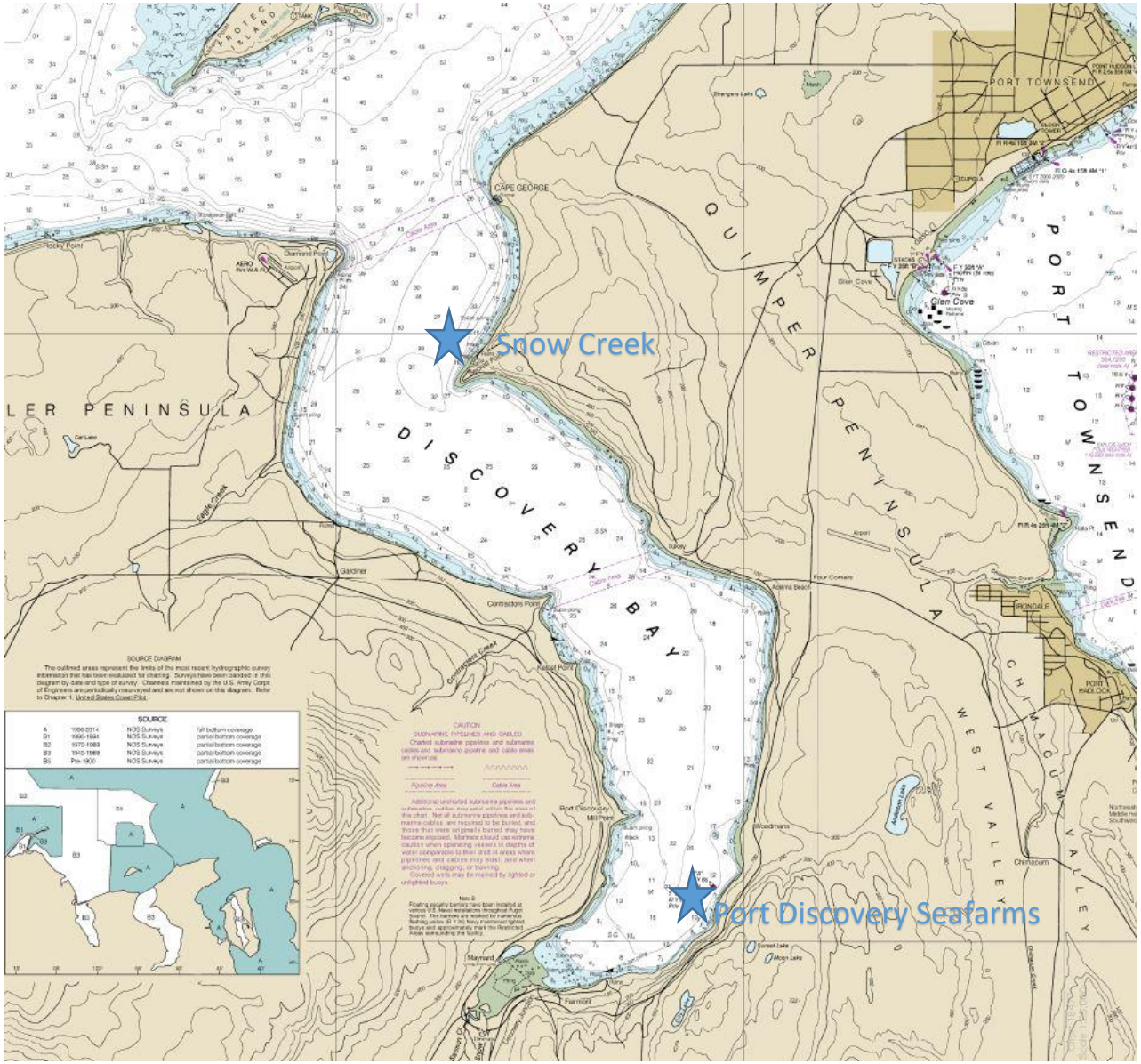
2.2.2 Problem Definition

The systematic planning process and DQOs for the overall study is documented the Data Quality Objective (DQO) Process for Port Discovery Seafarms (Appendix A). The overall objectives and associated problem statements are listed below:

1. The question to be answered is “Identify the cause(s) of toxicity to shellfish at Port Discovery Seafarms.” The observed toxicity may be due to biological, chemical or physical factors in the surface waters of Discovery Bay. It is also possible that a combination of factors is responsible for the observed toxicity. Because different life stages of oysters and other shellfish have differing sensitivities to stressors, it may be possible that toxicity to the different life stages of shellfish, or to the different species of shellfish, may be due to different causes. The study proposed is designed to evaluate these possibilities, although due to the nature of this investigation and limited and seasonal availability of some of the information needed to answer the above question, activities described in this QAPP may occur at different times.

The DQOs are summarized in Table 2-1 and detailed in Appendix A (Systematic Planning/Data Quality Objectives).

Figure 2-3 – Surface water sampling locations within Discovery Bay



2.3 Project Description (A6)

2.3.1 Description of Work Tasks

The work activities intended to be covered under this QAPP are summarized in Table 2-1 and include the following. Note that not all work activities are needed for chemical analysis of oyster shells and larval oyster toxicity tests:

- Collect a sufficient volume of Discovery Bay surface water for use in laboratory toxicity testing with larval Pacific oysters
- Collect a sufficient volume of reference area surface water from near successful oyster culture areas near the mouth of Discovery Bay for use in laboratory toxicity testing with larval Pacific oysters
- Collect adult Pacific oyster shells from animals affected by the mortality events at Port Discovery Seafarms, as well as Pacific oyster shells from other locations within Discovery Bay
- Manage and ship samples to MEL and/or using Scribe Project Manager.
- Analysis of samples by MEL.
- Perform laboratory water column 2-3-day (or until larval oysters have settled onto a solid substrate) toxicity tests with free swimming larval Pacific oysters
- Validation of sample results by EPA
- Evaluation of sample results according to the procedures described in Appendix A (Systematic Planning/Data Quality Objectives)

2.3.2 Project Schedule

The work is currently planned to occur in winter and spring of 2017. Specific dates cannot yet be assigned to the various tasks due to the experimentation and research required before methods to perform the various project tasks can be finalized. The overall project schedule is summarized as follows:

- Final QAPP approval –May 2017
- Mobilization of field crew and equipment – May – June 2017
- Surface water sampling at site locations – May to June 2017
- Demobilization – June 2017
- Sample analysis and validation (sample turn-around times for the analyses indicated in Table 2-1 range from 21 days to 8 weeks) – May July 2017
- Laboratory toxicity testing with Pacific oysters – Three types of toxicity tests are proposed, not including toxicity test method development.
    - Test with existing Discovery Bay surface water, collected Spring 2017, to identify if bay water is significantly different than reference bay to larval oyster toxicity
    - Potentially test with existing Discovery Bay surface water, with pH modification using CO_2 addition to modify pH and calcium carbonate mineral species
    - Potentially test with existing Discovery Bay surface water with ammonia additions, to determine if elevated NH3 is causing larval oysters to settle onto substrates prematurely
- Calculations and data reviews – April – June 2017

2.4 Quality Objectives and Criteria (A7)

2.4.1 Project Quality Objectives

Project-specific technical systematic planning has been carried out through the DQO process planning tool (EPA, 2006) to meet decision maker and data user needs for each activity. Appendix A presents the DQOs for Initial Tasks sampling activities.

The data needs as determined through the DQO process are presented in Table 2-2 (located at the end of this section). This table lists the specific analytes, data uses, data users, and needed analytical sensitivity. The selected analytical methodology and associated laboratory analytical reporting limits for oyster shell tissue are shown in Table 2-3. Toxicity testing quality objectives are defined in the test acceptability criteria for the toxicity test.

The needed detection limits (which are based on protective values for ecological exposure to surface water and measured metal concentrations in oyster shells from uncontaminated areas) and the analytical method reporting limits are compared in Step 5 of the DQOs (Appendix A). The target analytical method reporting limits (MRLs) are consistent with the needed limits. For most analytes, laboratory-specific method detection limits (MDLs) are expected to be below needed method reporting limits listed in Table 2-3. Where sample-specific method reporting limits are higher than metal concentrations typically found in uncontaminated oyster shells (indicated by bold font), the project team may not be able to make project decisions based on quantitative analytical chemistry data.

2.4.2 Measurement Performance Criteria

The QA objective of this plan is to identify procedures and criteria that will provide data of known and appropriate quality for the needs identified in Section 2.4.1. Data quality is assessed by representativeness, comparability, accuracy, precision, and completeness. These parameters, the applicable procedures, and level-of-effort are described in the following paragraphs.

The applicable quality control (QC) procedures and level-of-effort for assessing data quality are dictated by the intended use of the data and nature of the analytical methods. The intended use of the data from the oyster shell analyses is to determine if one or more metal concentrations in shells from Port Discovery Seafarms are elevated relative to oyster shells from areas without the observed toxicity at Port Discovery Seafarms. Analytical parameters, analytical methods, applicable reporting limits, analytical precision, accuracy, and completeness in alignment with needs identified in Section 2.4.1 are presented in Tables 2-3. Analytical methods and quality control procedures are further detailed in Section 3.

Following are definitions and levels of effort for the data assessment parameters (project criteria for these Data Quality Indicators are specified in Tables 2-3 and 2-4):

- **Sensitivity** is the capability of an analytical method to discriminate between measurement responses representing different levels of variable interest. Literature values of measured metal concentrations in uncontaminated oyster shells are listed in Table 2-3 to assist in developing actual method reporting limits. "Target" implies that final sample-specific reporting limits might be higher because of sample matrix effects. Sample-specific MDLs for the individual samples along with the MRLs will be reported in the final electronic data deliverable (Universal EDD), as defined in the EPA Region 10 Data Management Plan (2014). Some of the method reporting limits might be higher than the needed project criteria because of matrix effect, dilutions, preparation/digestion weight (solids) or because no practicable methodology for lower detection is available. Laboratory-specific MDLs are significantly below reporting limits. Where reporting limits for non-detects are

higher than the project criteria, the project team will use MRLs, as needed, for project decisions. The sample-specific MDL and MRL are provided in the lab EDD for project use, however all non-detect samples are reported at the MRL and qualified "U". If reported, values between the MRL and MDL are an estimate and will be qualified "J" for proper use.

- **Representativeness** is a measure of how closely the results reflect the actual concentration or distribution of the chemical compounds in the matrix samples. Sampling plan design in Appendix A, sampling techniques, and sample-handing protocols (e.g., for storage, preservation, and transportation) are discussed in Section 3 of this QAPP. The proposed documentation will establish that protocols have been followed and sample identification and integrity ensured.
- **Comparability** expresses the confidence with which one data set can be compared to another. Data comparability will be maintained using defined procedures and the use of consistent methods and consistent units. Actual MRLs will depend on the sample matrix and will be reported as defined for the specific samples.
- **Accuracy** is an assessment of the closeness of the measured value to the true value. For samples, accuracy of chemical test results is assessed by spiking samples and blanks with known standards and establishing the average recovery. For a matrix spike, known amounts of a standard compound identical to the compounds being measured are added to the sample. A quantitative definition of average recovery accuracy is given in Section 5.3. Accuracy is a combination of random error (precision) and systematic error (bias), introduced during sampling and analytical operations. Bias is the systematic distortion of a measurement process that causes errors in one direction, so that the expected sample measurement is always greater or lesser to the same degree than the sample's true value. The accuracy of measurement data will be determined by calculating the recoveries from the analysis of standard reference materials and laboratory and laboratory fortified samples (matrix spikes). Accuracy measurements will be carried out with a minimum frequency of 1 in 20 samples analyzed.
- **Precision** of the data is a measure of the data spread, when more than one measurement has been taken on the same sample. Precision can be expressed as the relative percent difference; a quantitative definition is given in Section 5.3. The level of effort for precision measurements will be a minimum of 1 in 20 samples.
- **Completeness** is a measure of the amount of valid data obtained from the analytical measurement system and the complete implementation of defined field procedures. The quantitative definition of completeness is given in Section 5.3. The target completeness objective will be 90 percent; the actual completeness might vary depending on the intrinsic nature of the samples and the ability to assess sample locations and collect field samples. The completeness of the data will be assessed during QC reviews.

2.5 Special Training/Certification (A8)

All project staff working on the Discovery Bay and Port Discovery Seafarms project will be trained in health and safety and follow requirements specified in the project's HSP. The HSP (Appendix D) describes the specialized training required for personnel on this project and the documentation and tracking of this training is also included in the HSP. All laboratory staff will follow the requirements of the R10 Laboratory Safety and Health Manual (EPA, 2012) and the Chemical Hygiene Plan (EPA, 2013).

2.6 Documents and Records (A9)

Project systematic planning through the DQO is documented in Appendix A of this QAPP. Required field documentation and records are described in Appendix B.

Laboratory documentation will be provided in accordance with methods and QA protocols listed in Sections 3.4 and 3.5 of this QAPP, the R10 DMP (EPA 2014) and with EPA Regional Laboratory-specific standard operating procedure (SOPs).

Overall project documentation will be prepared in accordance with the EPA Region 10 AES Program Plan (EPA, 2003a and b and updates) and the R10 DMP (EPA 2014).

Table 2-1
Summary of Sampling and Analysis Plan

Discovery Bay and Port Discovery Seafarms Sampling Initial Tasks QAPP

Sample Tier	Activity	Rationale for Activity	Target Areas	Target Media	Sample Design	Primary Sample Count	QA/QC Sample Count	Sample Depth and Basis	Target Analyte Suites	Basis for Study Activity and Target Analyte Suites
Initial analyses that can be performed between fall 2016 and spring 2017	Larval oyster toxicity testing, unmodified Discovery Bay surface water	Determine if larval oysters can survive, grow and settle onto a substrate when exposed to unmodified Discovery Bay surface water from vicinity of Port Discovery Seafarms	Discovery Bay, two locations. 1. Port Discovery Seafarms (area with observed toxicity. 2. Snow Creek Seafarms (reference area)	Surface water	Determine if toxicity to oysters occurs during a time of year when larval oyster culture normally does not occur at Port Discovery Seafarms, to determine if toxicity is seasonal or year-round	Three one liter bottles of seawater from Port Discovery Seafarms and Snow Creek Seafarms reference area. Number of bottles has been determined based on required sample volume to perform toxicity tests	Not applicable	1 meter below water surface, to avoid surface microlayer. May need to adjust during field sampling to simulate depth of water intake at Port Discovery Seafarms, and depth of oyster culture at Snow Creek Seafarms.	None Standard toxicity test analytes (salinity, temperature, dissolved oxygen, pH, ammonia, sulfide) measured at toxicity testing laboratory	Determine if Discovery Bay water collected during fall supports oyster survival, development and settling during time of year oyster culture generally not performed. Determine if larval toxicity is seasonal or occurs year-round.
	Larval oyster toxicity testing, Discovery Bay surface water with pH adjusted using CO_2 additions. Task within EPA Toxicity Identification Evaluation (TIE) protocol.	Determine if larval oysters can survive, grow and settle onto a substrate when exposed to acidified Discovery Bay surface water from vicinity of Port Discovery Seafarms, and the level of acidification needed to prevent settling or otherwise adversely affect larval survival. Test designed to simulate ocean acidification effects on larval oysters in Discovery Bay water	Discovery Bay, two locations. 1. Port Discovery Seafarms (area with observed toxicity. 2. Snow Creek Seafarms (reference area)	Surface water	Determine if toxicity to oysters occurs if surface water pH is reduced from ambient, to determine when acidification starts to adversely affect oyster larvae. Depending on the results of toxicity tests with unacidified Discovery Bay water, pH may need to be elevated to mitigate any observed toxicity in unmodified Discovery Bay water.	Multiple one liter bottles of seawater from Port Discovery Seafarms and Snow Creek Seafarms reference area. Number of bottles to be determined based on required sample volume to perform toxicity tests, minimum of three one liter bottles required per location	Not applicable	1 meter below water surface, to avoid surface microlayer. May need to adjust to simulate depth of water intake at Port Discovery Seafarms, and depth of oyster culture at Snow Creek Seafarms.	None Standard toxicity test analytes (salinity, temperature, dissolved oxygen, pH, ammonia, sulfide) measured at toxicity testing laboratory	Determine if ocean acidification within Discovery Bay water may be associated with larval oyster toxicity. If observed, should confirm with field monitoring in 2017.
	Adult Pacific oyster shell collection	Determine if metal concentrations in shells from Port Discovery Seafarms are elevated relative to those from other locations within Discovery Bay or a reference area (Dabob Bay)	Discovery Bay, Dabob Bay, including affected oysters from Port Discovery Seafarms	Adult Pacific oyster shells	Judgmental, opportunistic – will need to be limited to locations where oysters can be collected	Up to 10 grab samples, each grab sample consisting of both valves from 5-10 oysters	1 MS 1 MSD	Opportunistic sampling, cannot be defined in advance.	TAL metals (except total mercury, whose analysis is not required for this work)[a]	Oysters detoxify metals by depositing them in their shells, where they are biologically unavailable to oyster organs. Elevated Fe and Mn may be associated

Table 2-1
Summary of Sampling and Analysis Plan

Discovery Bay and Port Discovery Seafarms Sampling Initial Tasks QAPP

Sample Tier	Activity	Rationale for Activity	Target Areas	Target Media	Sample Design	Primary Sample Count	QA/QC Sample Count	Sample Depth and Basis	Target Analyte Suites	Basis for Study Activity and Target Analyte Suites
										with observed discoloration of adult shells.

[a] Laboratory-generated QC includes subsample replicates, MS/MSDs, and reference/control samples

MS = matrix spike
MSD = matrix spike duplicate
QA/QC = quality assurance/quality control
SU = sampling unit
TAL = target analyte list

TABLE 2-2
Data Needs and Uses

Matrix	Laboratory Analytical Suites	Field Data	Data Use	Data User	Needed Detection Levels
Pacific Oyster Shells	Total TAL metals. Mercury analysis NOT required.	Sample location coordinates Sample description and photographs	Characterize metals in shells. Oysters detoxify excess metals in water and diet by incorporating metals into a biologically unavailable form in their shells	Risk assessors, Chemists	Standard TAL detection limits. Many metals will be at concentrations well in excess of 1 mg/kg dry weight shell. Note that mercury analyses are not required for this work.
Laboratory toxicity test water	Most if not all of the below analytes can be measured by the toxicity testing laboratory. Temperature Dissolved oxygen Conductivity/Salinity pH Total alkalinity TIC pCO_2 Ammonia Sulfide	Not applicable	Identify the change in pH and other water chemistry parameters associated with increased mortality, decreased development and settling ability in free swimming larval Pacific oysters	Risk assessors, Chemists, Biologists, Toxicologists	Temperature – 0.1°C Dissolved oxygen – 0.1 mg/L Salinity – 0.1 ‰ pH – 0.01 pH unit Alkalinity – 0.1 mg/L Total inorganic carbon – 0.1 mg/L pCO_2 - 0.1 mg/L Ammonia – 0.01 mg/L Sulfide – 0.01 mg/L

TAL = target analyte list
TIC = total inorganic carbon
pCO_2 = partial pressure of carbon dioxide

TABLE 2-3
Oyster Shell Analytical Methods and Estimated Method Reporting Limits
TBD with Manchester Lab

Metal	Analytical Method TBD	Method Reporting Limit * (mg/kg dry wt.)
Al		200**
Sb		0.02
As		0.29
Ba		4.1
Be		<0.1
Cd		0.2
Ca		380,000
Cr		1.2
Co		0.2
Cu		0.6
Fe		313
Pb		3.8
Mg		1400
Mn		17
Ni		<1
K		80
Se		<0.3
Ag		0.04
Na		6500
Tl		<0.5
Zn		5.2

*Estimated method reporting limits (MRLs) are measured metal concentrations in uncontaminated oyster shells, listed for informational purposes only to assist in defining actual method reporting limits and method detection limits (MDLs)

**Metal concentrations in uncontaminated oyster shells taken from multiple literature citations, listed below:

Almeida, M.J., G. Moura, T. Pinheiro, J. Machado and J. Coimbra. 1998. Modifications in *Crassostrea gigas* shell composition exposed to high concentrations of lead. Aquatic Toxicology 40:323-334.

Darracott, A. 1986. Potential and problems in using shellfish as geochemical indicators in the marine environment. p. 309-326 in Thornton, I. and R. Howarth, eds. Applied Geochemistry in the 1980s. Graham & Trotman, Ltd., London, United Kingdom. 349 pp.

Pavlov, D.F., J. Bezuidenhout, M.V. Frontasyeva and Z.I. Goryainova. 2015. Differences in trace element content between non-indigenous farmed and invasive bivalve mollusks of the South African coast. American Journal of Analytical Chemistry 6:886-897.

SECTION 3

Data Generation and Acquisition (EPA Group B)

This section describes the sampling design; sampling methods; sampling handling and custody; analytical methods; quality control; instrument/equipment testing, inspection and maintenance; instrument/ equipment calibration and frequency, inspection/acceptance of supplies and consumables; nondirect measurements; and data management.

3.1 Sampling Design (Experimental Design) (B1)

The rationale for and the design for sampling activities are detailed in Appendix A, Data Quality Objectives.

3.2 Sampling Methods (B2)

Methods and protocols are described in Appendix B, Field Procedures. Additional method development will be required by MEL prior to analyzing shells for metals. The oyster grower will provide deformed adult oyster shells to EPA staff. Additionally,

3.3 Sample Handling and Custody (B3)

A sample is physical evidence collected from a potential hazardous waste site, the immediate environment, or another source. Because of the potential evidentiary nature of samples, the possession of samples must be traceable from the time the samples are collected until they are introduced as evidence. In addition to field notebooks, a number of documents are available for tracking sample custody.

EPA custody seals will be obtained from the RSCC in EPA's Region 10 Quality Assurance team. Standard chain-of-custody procedures will be used to maintain and document sample collection and possession. After sample packaging, the appropriate chain-of-custody form will be completed. Scribe software will be used for project data management and completing chain-of-custody documentation in accordance with the R10 Data Management Plan (DMP) (EPA, 2014).

Copies of the TR-COC, Scribe XML (*.xml) and Excel (*.xls) are submitted to the RSCC in accordance with the instructions for sample shipping and documentation per R10 requirements (2014 DMP). All Scribe project information, sample information, and documentation (labels/TR-COCs) must be completed according to the Region 10 DMP (2014). A separate unique Traffic Report (TR)/chain-of-custody will be created for each cooler shipped, documenting the specific contents and location of the associated cooler.

The following subsections summarize each element of sample handling and custody.

3.3.1 Chain-of-Custody

Because samples collected during any investigation could be used as evidence, their possession must be traceable from the time the samples are collected until they are introduced as evidence in legal proceedings. Chain-of-custody procedures are followed to document sample possession.

3.3.1.1 Definition of Custody

A sample is under custody if one or more of the following criteria are met:

- The sample is in a person's physical possession.
- The sample is in a person's view after being in his or her physical possession.
- The sample was in a person's physical possession and was then locked up or sealed to prevent tampering.
- The sample is kept in a designated secured area.

3.3.1.2 Field Custody

Only enough material to provide a good representation of the media being sampled will be collected. To the extent possible, the quantity and types of samples and sample locations are determined before the actual fieldwork is performed. As few people as possible should handle samples.

The field sampler is personally responsible for the care and custody of the samples collected until they are transferred or dispatched properly.

The PM determines whether proper custody procedures were followed during the fieldwork, and decides whether additional samples are required.

3.3.1.3 Transfer of Custody and Shipment

Samples are accompanied by a chain-of-custody record. When transferring samples, the individuals relinquishing and receiving the samples sign, date, and note the time on the record. This record documents custody transfer from the sampler, often through another person, to the analyst at the laboratory.

Samples are packaged properly for shipment and dispatched to the appropriate laboratory for analysis, with a separate chain-of-custody record accompanying each shipping container (one for each field laboratory if being used and one for samples shipped to the laboratory). Shipping containers will be sealed with custody seals for shipment to the laboratory. If used, courier service names and other pertinent information are entered in the "Received by" section of the chain-of-custody record. Given the proximity of Discovery and Dabob Bays to the EPA Manchester Laboratory, we anticipate that the EPA field crew would personally deliver samples to MEL if they are performing the chemical analyses of samples. The RSCC will be notified of shipment and the Scribe .xml file will be uploaded to the CLP Sample Management Office (SMO) Portal Web site on the day of shipment.

All shipments are accompanied by the chain-of-custody record identifying its contents. The original record and one copy accompany the shipment to the laboratory, and a second copy is retained by the PM. The Scribe .xml file is also emailed to the RSCC along with the R10 template custom view .xls file export.

It is anticipated that all samples will be delivered to the Manchester laboratory by the EPA staff who collected the samples. No FedEx, UPS or other commercial shippers are expected to be needed for this work.

3.3.1.4 Laboratory Custody Procedures

A designated sample custodian accepts custody of the shipped samples and verifies that the sample numbers match those on the chain-of-custody records. Pertinent information about shipment, pickup, and courier is entered in the "Remarks" section. The custodian then enters the sample numbers into a bound notebook. The laboratory custodian uses the sample identification number or assigns a unique laboratory number to each sample, and is responsible for ensuring that all samples are transferred to the proper analyst or stored in the appropriate secure area.

The custodian distributes samples to the appropriate analysts. Laboratory personnel are responsible for the care and custody of samples from the time they are received until the sample is exhausted or returned to the custodian. The data from sample analyses are recorded on the laboratory report form.

When sample analyses and necessary QC checks have been completed in the laboratory, the unused portion of the sample will be retained until specific written permission for disposal is received from EPA. The unused portion of the sample will then be disposed of properly. All identifying sample tie tags, data sheets, and laboratory records are retained as part of the documentation. Sample containers and remaining samples are disposed of by the laboratory in compliance with all federal, state, and local regulatory requirements.

3.3.2 Custody Seals

Custody seals will be placed on coolers during transport of samples to the laboratory. The seals will be placed on two sides of the lid (one in front, and one on the side) and covered with tape to prevent

inadvertent breaking of the seals. To prevent the opening of coolers during shipment and to ensure that the samples remain sealed under custody until arrival at the lab additional large liner bag (drum liner type) inside around entire contents of cooler, tied tightly closed and secured with additional custody seal will also be used.

3.3.3 Field Notebooks

A bound field notebook will be maintained by each sampling FTL to provide a daily record of significant events, observations, and measurements during field investigations. All entries will be signed and dated. The notebook will be retained by each agency as a permanent record, and copies of field notes from each sampling event will be maintained by EPA.

These notebooks are intended to provide sufficient data and observations to enable participants to reconstruct events that occurred during the project, and to refresh the memory of the field personnel, if required. Field data collected in field notebooks will be entered electronically for upload and final storage.

All project data as defined in the R10 DMP and including available field collection data will be documented in Scribe and uploaded to Scribe.net for archival in this EPA database.

3.3.4 Corrections to Documentation

All original data recorded in field notebooks and field data forms will be written in waterproof ink, unless prohibited by weather conditions. None of these accountable serialized documents is to be destroyed or thrown away, even if they are illegible or contain inaccuracies that require a replacement document.

If an error is made on an accountable document, personnel may make corrections simply by drawing a single line through the error and entering the correct information. The erroneous information should not be obliterated. Any subsequent error discovered on an accountable document should be corrected by the person who made the entry. All subsequent corrections must be initialed and dated.

3.4 Analytical Chemistry Methods (B4)

Project analytes, methods and target laboratory method reporting limits are listed in Tables 2-3. The samples are expected to be analyzed by MEL.

We are unaware of any available standard analytical methods which fully describe the sample digestion, processing and subsequent chemical analysis of oyster shells. Thus, we cannot provide MEL with specific procedures to follow. The two methods which will need to be developed with the assistance of the EPA Manchester lab are the digestion of oyster shells into a liquid which can then be chemically analyzed, and the procedures and methods needed to minimize or eliminate analytical interferences of the required metal analyses.

3.4.1 Oyster Shell Digestion Procedure

The following procedure should solubilize Pacific oyster shells. Note that analysis of oyster shell soft tissue is not required, only the shells are to be analyzed.

1. If oysters have not been received in a shucked condition, remove all soft tissue from the interior of the oyster shells. EPA-Seattle staff can perform this work if desired by MEL.
2. Rinse the exterior and interior of the shells with a stiff brush to remove any remaining attached material to the interior and exterior of the shells
3. Dry the shells in a drying oven at 60°C for 24 hours to remove remaining water.
4. For shells with deformities and/or discoloration, as best as possible, remove the deformed/discolored part of the shell from the remainder of the shell, and retain both parts of the shells for chemical analyses. The deformed/discolored parts of the shells are to be considered a separate sample for analysis from the remainder of the shell. Oyster shells can be cut with either a

lapidary saw or a dremel with a carbide cutting wheel. Wear respiratory protection during the cutting, as the fine shell dust is sharp edged, can be inhaled and cause physical damage to lung tissue. NOTE: EPA-Seattle staff will assist with this separation process.

5. Grind the oyster shells (or sections of shells if analyzing deformed/discolored part of shell) to a fine powder with a mortar and pestle, and /or methacrylate ball mill. An aliquot of the entire shell can be ground if grinding the entire shell will result in too large a sample mass to readily digest and analyze.
6. Weigh the dried, ground oyster shells (or shell subsample if the whole shell will not be digested), and record the dry weight of each sample.
7. Oyster shells will be digested following EPA Method 3050B.

3.4.2 Oyster Shell Metals Analysis

Metals in shells will be analyzed using ICP. Given the high salt content of the shell digestate (calcium carbonate primarily, roughly 38% of the shell by weight is calcium) it is likely that some procedures to minimize or eliminate analytical interferences will be required before the TAL metal analyses can be completed. This is an area where need to identify the best procedure(s) to reduce interferences. Fortunately, given the large sample mass of most of the oyster shells, there should be sufficient mass to identify the appropriate methods. Table 2-3 lists measured metal concentrations in uncontaminated Pacific oysters (*Crassostrea gigas*) and Eastern oysters (*C. virginica*). Method reporting limits for the metals at lower concentrations (all metals except Ca, Mg, K, Na) should not need to be appreciably lower than the values given in Table 2-3.

3.5 Laboratory Toxicity Test Methods

An initial toxicity test experiment with setting larval Olympia oyster (*Ostrea lurida*) larvae has already been successfully completed at MEL. A detailed protocol of this procedure, with modifications learned during the initial toxicity test will be employed to test Pacific oyster is Appendix E of this QAPP. The general outline of the procedure is discussed below.

Pacific oysters and blue mussels are both commonly used species in marine toxicity testing procedures to evaluate contaminants in both surface water and sediments. Although there are published EPA and ASTM standard toxicity test methods for larvae of both species, the published larval toxicity test protocols only evaluate effects on larvae from fertilization to formation of D-shaped larvae (i.e. initial stage of shell formation), the first 24 hours (oyster) to 48 hours (blue mussel) after fertilization. This test duration and lifestage does not represent the full development period of larval Pacific oyster from fertilization to setting on a substrate, which takes approximately 20 days, depending on water temperature and nutritional status of the larvae.

In order to determine if Discovery Bay water is toxic to larval shellfish, we will obtain larval Pacific oysters from a local supplier approximately three days before the larvae set on a substrate. The larvae will be exposed several types of water. Laboratory control water will be filtered and UV light treated seawater obtained from the NOAA-Manchester laboratory, which is drawn from Little Clam Bay. Reference water (i.e. water from a field location as similar as possible to the test water, but where oyster toxicity has not been observed) will be unmodified Dabob Bay water. Pacific oysters have been successfully reared on this reference water during the time when Discovery Bay water elicited toxicity to oysters. Unmodified Discovery Bay water from the vicinity of Port Discovery Seafarms will be the test water. This water will be tested during early spring to see if bay water collected during the spring also elicits toxicity (the observed toxicity at Port Discovery Seafarms has occurred during the summer of 2015). If bay water collected during the early spring does not elicit toxicity, the test may be repeated during summer when toxicity was initially observed, at times when salmon habitat restoration activities occur, or when diatom or other algal blooms occur in Discovery Bay.

Other suites of toxicity tests will be performed on Discovery Bay water with various modifications of the water quality, in an effort to identify physical or chemical parameters that may be eliciting the observed toxicity. These type of sample modifications, generally termed Toxicity Identification Evaluation (TIE) studies, are designed to sequentially evaluate whether physical or conventional contaminants (e.g. suspended solids, pH), ammonia, metals or organic compounds are associated with toxicity. Given the known occurrence of low pH values having adversely affected other oyster farms in the Pacific Northwest, the usual TIE protocol (EPA 1992, EPA 1991) will be modified somewhat to account for testing with Pacific oysters of an age ready to settle onto a substrate.

The modifications will consist of using Pacific oyster larvae approximately 18 days post fertilization old as test organisms. This age larvae should settle onto substrates within 2-3 days after test initiation. Larvae will be fed with the marine alga *Isochrysis galbana* throughout the toxicity test. Discovery Bay water pH modification will be the first sample modification tested. The pH will be changed by bubbling various amounts of carbon dioxide through exposure water to reduce the pH of marine water from its normal range of pH 8.0 – 8.2. If acidification does not result in the observed larval oyster toxicity, the next modification to be tested will be addition of increasing levels of ammonia to 18 day post fertilization larval Pacific oyster. If ammonia concentrations at those similar to those in Discovery Bay do not elicit toxicity, the next two stages of the TIE will be to remove metals from solution, then remove organic chemicals from water. This TIE approach should identify either acidification, ammonia, or a broad category of metals and organic chemicals as a factor associated with the observed oyster mortality. If none of the TIE modifications elicit toxicity at concentrations known or which could possibly exist in Discovery Bay, this will be considered evidence that chemical contamination is unlikely to be the cause of toxicity observed in larval oysters.

Toxicity test methods and test acceptability criteria will be modified from the EPA (1995) *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms*, to account for the different life stage of Pacific oyster to be tested in these investigations.

3.6 Quality Control (B5)

3.6.1 Field Quality Control Procedures

QC requirements related to the sample collection process (i.e., sample design, sampling procedures, and field QC samples) are summarized in Appendix B.

The QC samples will be collected immediately following collection of normal samples and using the same procedures as the collection of the normal sample.

Instrument calibration and standards for field meters (temperature, dissolved oxygen, pH, salinity) follow MEL SOPs for each meter.

3.6.2 Laboratory Quality Control Procedures

Laboratory QC procedures will include the following:

- Analytical methodology and QC according to methods listed in Tables 2-3.
- Instrument calibration and standards as defined in the methods listed in Tables 2-3 and laboratory (MEL) SOPs.
- Laboratory blank measurements at a minimum 5 percent or 1-per-batch frequency.
- Accuracy and precision measurements at a minimum of 1 in 20, 1 per set.
- Data reduction and reporting according to the methods listed in Tables 2-3.
- Laboratory documentation per MEL standard operating procedure.

3.7 Instrument/Equipment Testing, Inspection, and Maintenance (B6)

Field instrument testing, inspection and maintenance will be recorded in field notebooks. Preventative maintenance is performed according to the procedures described in the manufacturer's instrument manuals, if applicable, including lubrication, cleaning, and the frequency of such maintenance. Instrument downtime is minimized by keeping adequate supplies of all expendable items, where expendable means an expected lifetime of less than 1 year. These items include batteries, oil, and cables. Preventative maintenance for field equipment (e.g., water level meter, pressure transducers, and the water quality meter) will be conducted in accordance with procedures and schedules outlined in the particular model's operation and maintenance handbook.

3.8 Instrument/Equipment Calibration and Frequency (B7)

3.8.1 Field Calibration Procedures

Planned instruments used in the field include global positioning system (GPS), a photoionization detection (PID), and a multi-parameter water quality meter. The GPS is calibrated and/ or checked by the manufacturer and should not require any adjustment or calibration in the field; however calibration checks will be carried out by the field team as needed. The PID and the multi-parameter water quality meter will be calibrated each day, prior to use and be verified at the end of each day's use. Any instrument deviations from the calibration solution should be recorded in the field notebook. Instrument adjustments will be in accordance with procedures and schedules outlined in the particular instrument's operations and maintenance manual.

Scheduled periodic calibration, if any, of testing equipment does not relieve field personnel of the responsibility of employing properly functioning equipment. If an individual suspects an equipment malfunction, the device must be removed from service and tagged so that it is not inadvertently used, and appropriate personnel notified so that a recalibration can be performed or a substitute piece of equipment can be obtained. Equipment that fails calibration or becomes inoperable during use will be removed from service and either segregated to prevent inadvertent use or tagged to indicate it is out of calibration. Such equipment will be repaired and satisfactorily recalibrated. Equipment that cannot be repaired will be replaced.

Results of activities performed using equipment that has failed recalibration will be evaluated. If the activity results are adversely affected, the results of the evaluation will be documented, and the PM and data users will be notified.

3.8.2 Laboratory Calibration Procedures

Laboratory calibration procedures are specified in the methods referenced in Tables 2-3and in the laboratory's SOPs.

3.9 Inspection/Acceptance of Supplies and Consumables (B8)

Supplies and consumables will be acquired and inspected in accordance with acquisition specifications upon receipt.

3.10 Non-direct Measurements (B9)

As documented in Step 3 of the DQO process for each problem statement (Appendix A), data collected during this study may be augmented with existing data. This existing data may include information on algal blooms obtained by Ecology, and information on water circulation patterns, meteorology patterns and events in the vicinity, to determine whether it is possible that material released during salmon restoration activities in other portions of Discovery Bay are or could be transported to the location of Port Discovery Seafarms. If chemical contaminant data from other sources were to come to our attention, EPA will

document the usability of such existing data sets and describe how they may or may not be used in making decisions about the nature and extent of contamination, risk, and/or the sources of toxicity.

3.11 Data Management (B10)

All data for all parameters will undergo two levels of review and validation, as applicable: (1) at the laboratory, and (2) outside the laboratory as described in Section 5. Following receipt of reviewed and validated data, data will be uploaded to Scribe.net to facilitate data access, queries, and report preparation. All analytical data generated by the EPA Manchester Laboratory is entered into Scribe, which is also used to generate data reports of analytical results. Scribe software will be used to document and manage sample data for collection, custody, locational information, shipment, monitoring/field results, final validated lab results, and other data associated with this QAPP in accordance with the R10 DMP (2014). The Scribe project file is periodically and at project completion published to Scribe.net for EPA data warehousing.

At project completion, the Scribe file is published to Scribe.net. The backup file (.bac) is provided to the EPA RSCC. If any GIS documents are produced for this project they shall be provided to the EPA RPM and RSCC in accordance with the R10 GIS Data Deliverable Guidance for R10 Lead Projects and R10 Contractors (4/2014). The site-specific data management plan is provided as Appendix C.

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SECTION 4

Assessment and Oversight (EPA Group C)

This section describes assessment, oversight, and reports to management.

4.1 Assessments and Response Actions (C1)

The QAO, RTL, and PM will monitor the performance of the QA procedures. If problems arise or the EPA Coordinator directs the PM accordingly, the EPA QAO and PM will conduct field audits. Field audits may be scheduled to evaluate the following:

- Execution of field measurements
- Whether field information gathering procedures were properly implemented
- Execution of sample identification, chain-of-custody procedures, field notebooks, sampling procedures, and field measurements
- Whether trained personnel staffed the sample event
- Whether equipment was in proper working order
- Availability of proper sampling equipment
- Whether appropriate sample containers, sample preservatives, and techniques were used
- Whether sample packaging and shipment were appropriate
- Whether QC samples were properly collected

Preparation of sample aliquots for analysis will be conducted at EPA MEL. Chemical analyses of samples will be carried out at EPA MEL. The RSCC, residing at EPA's Environmental Services Unit (ESU), will be responsible for coordinating and scheduling analytical services from MEL.. For MEL, QA oversight is provided by the laboratory's QA Coordinator. In addition, onsite audits or performance evaluation samples will be administered by the EPA Regional QAM, as necessary. Audits will be followed up with an audit report prepared by the reviewer. The auditor will also debrief the laboratory or the field team at the end of the audit and request that the laboratory or field team comply with the corrective action request.

If QC audits result in detection of unacceptable conditions or data, the PM will be responsible for developing and initiating corrective action. The Project Coordinator will be notified if non-conformance is of program significance or requires special expertise not normally available to the project team. In such cases, the PM will decide whether any corrective action should be pursued. Corrective action could include the following:

- Recollecting field data if practicable
- Evaluating and amending field data collection procedures
- Reanalyzing samples if holding time criteria permit
- Resampling and analyzing
- Evaluating and amending sampling and analytical procedures
- Accepting data acknowledging a level of uncertainty

All corrective actions will be documented in a field logbook.

4.2 Reports to Management (C2)

The PM or Project Coordinator may request that a QA report be made to the Project Coordinator on the performance of sample collection and data quality. The report will include the following:

- Assessment of measurement data accuracy, precision, and completeness
- Results of performance audits
- Results of systems audits
- Significant QA problems and recommended solutions

Progress reports, prepared as needed, will summarize overall project activities and any problems encountered. QA reports generated on sample collection and data quality will focus on specific problems encountered and solutions implemented. Alternatively, in lieu of a separate QA report, sampling and field measurement data quality information may be summarized and included in the final reports. The objectives, activities performed, overall results, sampling, and field measurement data quality information for the project will be summarized and included in the final reports along with any QA reports.

A field sampling report listing the dates of field activities, information collected, samples collected, sample locations, field duplicates, and dates of sample collection and shipment will also be generated to support the data validation activities.

SECTION 5

Data Validation and Usability (EPA Group D)

This section introduces the concepts of data review, verification, and validation; describes verification and validation methods; and explains reconciliation with user requirements.

5.1 Data Review, Verification, and Validation (D1)

The data generated by the regional EPA laboratory (MEL) is reviewed and verified internally at MEL and validation qualifiers are applied as needed; MEL data review is considered equivalent to a Stage 4 (S4VM). The stage of data validation as explained below will be included in the data validation report. All data is reported in the R10 EDD format, also known as the EPA national Universal EDD, as defined in the 2014 DMP.

5.2 Verification and Validation Methods (D2)

Initial laboratory analytical data reduction, validation, and reporting at the laboratory will be performed as described in the MEL laboratory-specific SOPs. Data validation performed for EPA laboratory results are labeled with a level-of-effort "Stage" identification in accordance with *Guidance for Labeling Externally Validated Laboratory* Analytical *Data for Superfund Use* (EPA, 2009b). Standardized terminology for identification of data validation is designed to help increase national consistency and improve communication and understanding about the nature of verification and validation conducted on laboratory analytical data. An in-depth definition of each data validation stage label can be found in Appendix A of the cited EPA guidance document.

Independent data validation by EPA or their designee will follow EPA guidance as applicable to method QC parameters (e.g., ASTM methods used for the Pacific oyster toxicity testing). An equivalent level of effort as prescribed in the guidance will be implemented.

5.3 Reconciliation with User Requirements (D3)

Laboratory analytical data obtained will be reconciled with the requirements specified in Table 2-2. Assessment of data for precision, accuracy and completeness will be performed in accordance with the quantitative definitions in the following sections.

The data will also be evaluated as described in Step 5 of the DQOs for each problem statement in Appendix A.

5.3.1 Precision

If calculated from duplicate measurements, use the following equation:

$$RPD = \frac{(C_1 - C_2) \times 100\%}{(C_1 + C_2) / 2} \quad (1)$$

Where:

RPD	=	relative percent difference
C_1	=	larger of the two observed values
C_2	=	smaller of the two observed values

If calculated from three or more replicates, use relative standard deviation (RSD) rather than the RPD, as follows:

$$RSD = (s / \bar{y}) \times 100\% \quad (2)$$

Where:

RSD = relative standard deviation
s = standard deviation
$\bar{y}$ = mean of replicate analyses

Standard deviation, s, is defined as follows:

$$S = \sqrt{\sum_{i=1}^{n} \frac{(y_i / \bar{y})^2}{n-1}} \quad (3)$$

Where:

s = standard deviation
y_i = measured value of the i^{th} replicate
$\bar{y}$ = mean of replicate analyses
n = number of replicates

5.3.2 Accuracy

For measurements where matrix spikes are used, use the following:

$$\%R = 100\% \; x\left[\frac{S-U}{C_{sa}}\right] \quad (4)$$

Where:

%R = percent recovery
S = measured concentration in spiked aliquot
U = measured concentration in unspiked aliquot
C_{sa} = actual concentration of spike added

For situations where a standard reference material (SRM) is used instead of or in addition to matrix spikes, use the following:

$$\%R = 100\% \; x\left[\frac{C_m}{C_{sm}}\right] \quad (5)$$

Where:

%R = percent recovery
C_m = measured concentration of SRM
C_{sm} = actual concentration of SRM

5.3.3 Completeness (Statistical)

Defined as follows for all measurements:

$$\%C = 100\% \; x\left[\frac{V}{T}\right] \quad (6)$$

Where:

%C = percent completeness
V = number of measurements judged valid
T = total number of measurement

Appendix A – Data Quality Objective (DQO) Process for Discovery Bay

Data Quality Objective (DQO) Process for Port Discovery Seafarms

The Data Quality Objective (DQO) Process (EPA 2006) is a seven step systematic planning process used by EPA to help design studies that acquire environmental data that will be used in decision making. In this case, the decision to be made is to determine the cause or causes of oyster mortality events that occurred at Port Discovery Seafarms in the summer of 2015. As stated in EPA (2006) the DQO Process "is used to develop performance and acceptance criteria (or data quality objectives) that clarify study objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions." Although not all details of a field sampling and monitoring plan are provided in this document, it does utilize the DQO process to define the types of data needed to evaluate and identify the causes of the observed oyster mortality.

1. **State the problem**
   1.1. ***Description of the problem.*** Port Discovery Seafarms, a Pacific oyster (*Crassostrea gigas*) and Manila clam (*Venerupis philippinarum*, formerly *Ruditapes philippinarum*) floating aquaculture facility located on the southeastern shoreline and adjacent waters of Discovery Bay, Jefferson County, Washington, suffered mortality events in July and August of 2015. A visible plume in Discovery Bay surface water during this time coated the beach and equipment at the facility. The following adverse effects were among those observed on shellfish in association with the observed plume, according to a February 16, 2016 shellfish pathology report prepared by the firm AquaTechnics of Carlsborg, WA:

   - 1.5 mm size oyster seed stopped growing
   - Shells of exposed stock turned black
   - Oysters had developed a white deposit along the edge of their valves
   - 90% of oyster seed of a size less than 75 mm died
   - 20% of market sized oysters died
   - Surviving seed oysters had abnormal shells – both valves grew in concave downward direction
   - A ridge of white material formed inside the margin of the valves of the surviving oysters
   - Market sized manila clams became weak and suffered substantial mortality
   - No Manila clam seed was on the site during July and August 2015
   - Mussels on the site suffered 90% mortality

   The problem is to identify the cause(s) of shellfish mortality, reduced growth and shell deformities at Port Discovery Seafarms.

2. **Identify the goal of the study**
   2.1. ***Principal study question(s).*** The question to be answered is "Identify the cause(s) of toxicity to shellfish at Port Discovery Seafarms." As will be described in the next section,

the observed toxicity may be due to biological, chemical or physical factors in the surface waters of Discovery Bay. It is also possible that a combination of factors is responsible for the observed toxicity. Because different life stages of oysters and other shellfish have differing sensitivities to stressors, it may be possible that toxicity to the different life stages of shellfish, or to the different species of shellfish, may be due to different causes. The study proposed is designed to evaluate all of these possibilities.

2.2. ***Consider alternative outcomes or actions that can occur upon answering the question(s).*** The goal of this study is to identify the cause(s) of toxicity. It is not the goal of this study to propose operational, treatment or engineering solutions to the identified cause(s) of toxicity, although the information obtained may be of use in developing approaches that will eliminate the observed toxicity Brief descriptions of possible alternative outcomes (i.e. the alternative cause(s) of the observed toxicity) of this study are given below.

- ***Biological factors as the cause of toxicity.*** The Port Discovery Seafarms pathology report (AquaTechnics 2016) appears to rule out infectious disease or bacterial infection as a cause of the observed toxicity. There are other biological factors that may adversely affect shellfish. Domoic acid, a toxin produced by several species of diatoms, particularly members of the genus *Pseudo-nitzschia*, is the cause of amnesic shellfish poisoning in humans who consume shellfish containing domoic acid. *Pseudo-nitzschia* were found in Discovery Bay in 2015 (Peninsula Daily News, July 4, 2015), while domoic acid has historically been detected in multiple shellfish species collected from Discovery Bay (Bills et al. 2006). One concern with domoic acid toxicity to Pacific oysters is described in a study by Jones et al. (1995), who observed that Pacific oyster exposure to domoic acid at a concentration of 536 µg/L resulted in a pH reduction of oyster hemolymph (the shellfish analogue to blood in vertebrates) from 7.35 to 6.79. If such a pH reduction from slightly basic to slightly acidic were to occur in larval mussels during the initial stages of shell formation, it could result in an inability to form shells, with subsequent larval mortality. Because of this potential, biological causes of the observed toxicity to shellfish cannot be discounted at this time. Another possible biological cause of the observed larval oyster mortality may simply be receipt by Port Discovery Seafarms of a suboptimal set of seed from its supplier, resulting in a failure of the larval oysters to grow and survive in the water quality conditions found in Discovery Bay. Poor larval development at Port Discovery Seafarms could also be a result of exposure of larval oysters to elevated partial pressure of carbon dioxide (pCO_2) in seawater at the supplier before receipt of larvae by Port Discovery (see a more detailed discussion of the potential for ocean acidification effects under physical factors as the cause of toxicity).
- ***Chemical factors as the cause of toxicity.*** Multiple chemical contaminants are known to cause shell deformations, as well as effects on survival, growth and reproduction of Pacific oysters and other shellfish. As correctly noted in the AquaTechnics (2016) pathology report, tributyltin has been demonstrated to cause shell deformation and other toxic effects on Pacific oysters. Other chemical contaminants shown to have caused shell deformities in Pacific oysters include polycyclic aromatic hydrocarbons (PAHs, Geffard et al. 2003); and cadmium, copper and lead (Fichet et al. 1998). Discoloration

of shellfish shells, particularly a shift to either orange or black exteriors on shells, has been attributed to deposition of iron on the shells. Hydrogen sulfide in anaerobic sediment can react with iron to form iron sulfide, which if subsequently deposited on bivalve shells results in a black coloration. Iron in aerobic sediments can react with oxygen to form ferric oxide, which when deposited in bivalve tissues or on the shells imparts a yellow or orange color to the animals. Ammonia, specifically the NH_3 chemical form that becomes more common in surface water as the pH becomes more basic above pH 8, can induce free swimming larval oysters to settle out of the water column before the larvae are fully developed and ready to attach to a substrate (Coon et al. 1990). Because of these potentials, chemical causes of the observed toxicity to shellfish cannot be discounted at this time.

- ***Physical factors as the cause of toxicity.*** It is well documented within the greater Puget Sound/Strait of Juan de Fuca/Strait of Georgia area that ocean acidification, nutrient upwelling and salinity variations have adversely affected shellfish aquaculture facilities (Feely et al. 2012. *Scientific Summary of Ocean Acidification in Washington State Marine Waters*). Fossil fuel combustion releases carbon dioxide (CO_2) to the atmosphere. When absorbed by seawater, CO_2 combines with water to release hydrogen ions, resulting in increased acidity (i.e. a reduction in pH). Elevated CO_2 in seawater also changes the chemical forms of inorganic carbon present, including a reduction in carbonate anion (CO_3^{-2}) concentration. Carbonate combines with the calcium present in seawater to form one of several mineral forms of calcium carbonate ($CaCO_3$): amorphous calcium carbonate, aragonite, low-magnesium calcite or high-magnesium calcite. The relative proportions of these different mineral forms of calcium carbonate are dependent on various properties of seawater, including pH, temperature, salinity, and partial pressure of carbon dioxide (pCO_2). The aragonite form of $CaCO_3$ is more water soluble than calcite under the same water chemistry conditions, and also dissolves more readily as seawater becomes increasingly acidic. Free swimming Pacific oyster larvae initially form their shells out of aragonite, but switch over to forming their shells from the less soluble calcite once they settle and become attached to a substrate. Reduced seawater pH from ocean acidification results in undersaturation of aragonite. For animals such as the free swimming larvae of Pacific oysters that precipitate aragonite during shell formation, this undersaturation requires the animal to expend more energy than it normally would to obtain sufficient aragonite to form its shell, resulting in adverse effects on larval oyster development, growth and ultimately survival. Ocean acidification has already been implicated in larval Pacific oyster mortality at both the Taylor facility in Dabob Bay, immediately to the east of Discovery Bay, and also at the Whiskey Creek facility in Oregon, with symptoms similar to those observed at Port Discovery Seafarms. Given the similarity in the observed toxicity of larval oysters at Discovery Bay to the mortality events at Taylor and Whiskey Creek, physical toxicity to oyster larvae associated with ocean acidification cannot be discounted at this time.

2.3 <u>Identify decision statements:</u> Information derived from this investigation should provide answers to the following decision statements.

- Can the observed toxic effects on oysters be recreated in a laboratory setting

- Are the same factors eliciting toxicity in both larval and adult oysters
- What are the factors that elicit the same toxicity observed *in situ* at Port Discovery Seafarms oysters
- Are chemical contaminant concentrations in surface water responsible for the observed *in situ* toxicity
- Are chemical contaminant concentrations in solid materials, either in sediments where release to overlying water has occurred, or in the visible plumes and precipitates observed concurrent with the *in situ* toxicity responsible for the observed toxic effects on oysters
- Are biological factors such as algal toxins or unhealthy stock animals responsible for the observed *in situ* toxicity
- Are physical factors associated with ocean acidification responsible for the observed *in situ* toxicity

3. Identify information inputs

3.1. ***Identify types and sources of information needed to resolve the decision statements***. The following information inputs are required for the three categories of potential stressors identified (i.e. biological, chemical and physical stressors). **NOTE THAT ONLY SURFACE WATER FOR TOXICITY TESTING AND OYSTER SHELLS FOR METAL ANALYSES WILL BE COLLECTED UNDER THIS QAPP.** The other possible sources of toxicity to oysters (e.g. algal toxins, sediment contaminants) are described only for completeness, as they have been identified as potential sources of the oyster toxicity within this DQO statement.

- ***Biological factors and stressors***
    - Analysis of surface water and adult oyster soft tissues for biologically generated toxins known to adversely affect shellfish, including domoic acid.
    - Laboratory toxicity tests of larval oysters from multiple suppliers reared in site water from Port Discovery Seafarms, to determine if the source of larval oysters affects their development and survival in Discovery Bay water. These studies should be performed for a 10-day duration (or to time of settlement on a substrate), not the 48-hour duration called for in the Puget Sound Estuary Program (1995) or ASTM (2012) bivalve larval development toxicity test protocol.
    - Biological evaluation of the plume of material that coated the beach and equipment at Port Discovery Seafarms, to determine if the plume contains elevated counts of algae (e.g. *Pseudo-nitzschia*) that release biological toxins known to be harmful to shellfish.
    - Toxicity testing is a major component of the needed information to identify the cause(s) of the observed effects on oysters. To meet the goals of this investigation identified in Step 2 of this DQO process, it is likely that multiple types of toxicity tests will need to be performed. The initial tests to be performed s will involve the development and survival of larval oysters under different pH conditions. This is because the observed symptoms of mortality in larval oysters (but not necessarily adult oysters) at Port Discovery Seafarms are remarkably similar to those observed at other shellfish production facilities in the Pacific

Northwest subjected to ocean acidification. Variation of physical factors such as pH and suspended solids is also the first step of an EPA (1991, 1992, 1993 for water column TIEs, 2007 for sediment TIEs) tiered toxicity testing protocol known as Toxicity Identification Evaluation (TIE). TIE toxicity tests perform a sequence of manipulations on samples, such as adjustment of sample pH, aeration, EDTA additions, and other manipulations to narrow down the possible causes of toxicity in samples. The toxicity testing sequence stops when the cause of toxicity has been identified.

- ***Chemical factors and stressors***
    - Chemical analysis of sediments from a range of locations within Discovery Bay, including from both Port Discovery Seafarms and the locations of ongoing and historical salmonid habitat improvement activities, to measure concentrations of chemicals known to elicit toxicity to shellfish, including those that cause shell deformation. In addition to analysis for toxic or hazardous substances, analyses for more conventional parameters such as ammonia, sulfide, and the ratio of acid volatile sulfides to simultaneously extractable metals (AVS:SEM) should also be performed.
    - Chemical analysis of surface water for toxic and hazardous substances, as well as for conventional parameters such as ammonia and nutrients
    - Chemical analysis of adult Pacific oyster shells for metals, particularly shells from oysters which died during the mortality events. Shellfish deposit excess metals to which they are exposed in water into their shells as a form of metal detoxification. Elevated metal concentrations in shells compared to metal levels in shells from healthy adults is a line of evidence that would support toxicity from metals to Discovery Bay oysters.
    - Chemical analysis of precipitates found in various locations and on various equipment at Port Discovery Seafarms. These precipitates, apparently not observed prior to the oyster toxicity events, may contain elevated concentrations of contaminants associated with the observed toxicity.
    - If possible to obtain sufficient mass, chemical analysis of the abnormally colored material (e.g. black, yellow/orange) found on and which can be flaked off of the exterior of shellfish shells.
- ***Physical factors and stressors***
    - Review water circulation patterns and meteorology patterns and events in the vicinity, to determine whether it is possible that material released during salmon restoration activities in other portions of Discovery Bay are or could be transported to the location of Port Discovery Seafarms.
    - Monitoring of Discovery Bay surface water at multiple locations for parameters that are indicative of sub-optimal conditions for oyster development and survival, including dissolved oxygen, salinity, suspended solids, and water temperature fluctuations.
    - Monitoring of Discovery Bay surface water at multiple locations for parameters indicative of ocean acidification. This monitoring is more detailed and elaborate than simple monitoring of water column pH. Specifically, the concentrations of

the various chemical forms of dissolved inorganic carbon (DIC) must be known, as they are directly related to the ability of shellfish, both larvae and adults, to form shells. Dissolved inorganic carbon consists primarily of the following chemical species: carbonate (CO_3^{-2}), bicarbonate (HCO_3^-), carbon dioxide (CO_2), and the mineral forms of calcium carbonate ($CaCO_3$): amorphous calcium carbonate, aragonite, low-magnesium calcite and high-magnesium calcite. In order to know the concentration of each chemical form of DIC present in seawater, at least two of four following analyses must be performed: DIC, pH, total alkalinity and pCO_2. When combined with measurements of water temperature and salinity, the concentration of the other two analytes can be calculated. In the most detailed studies of ocean acidification, the concentration of two additional analytes: phosphate and silicic acid have also been measured (Haigh et al. 2015). Analytical methods for these parameters and the calculations needed to determine concentrations of parameters not measured are presented in Feely et al. (2008).

4. Define the boundaries of the study

4.1. ***Define the target population of interest and its relevant spatial boundaries.*** The target populations are the shellfish populations (primarily Pacific oyster) in the vicinity of the Port Discovery Seafarms site in Discovery Bay. Because other locations within Discovery Bay may be the source(s) of factors that are adversely affecting shellfish production at Port Discovery Seafarms, other locations within Discovery Bay will need to be sampled and monitored. With the possible exception of uncontaminated reference areas with successful shellfish culture and growth outside of Discovery Bay, such as Dabob Bay, no locations outside of Discovery Bay are anticipated to be sampled or monitored during this study.

5. Develop the analytic approach

5.1. ***Specify appropriate population parameters for making decisions.*** This step involves deciding the statistical population parameter (e.g., mean, median, percentile or maximum detected value) considered to be important to make inferences about the target population. For the statistical comparisons anticipated for this work, comparison of measures of central tendency (e.g. sample or site means) for samples from Discovery Bay and reference areas or control samples will be performed. Specific statistical procedures to be used are discussed in Section 6.1.

Choose a workable action level and generate an "if…then…else" decision rule to evaluate study results. Toxicity tests have test acceptability criteria, specific for each toxicity test species and test protocol, which must be met before results are considered useable in a decision making context. Acceptable toxicity test results are usually evaluated with both an absolute magnitude of adverse effect and a statistically significant increase in sample toxicity compared to reference or control sample results to identify samples that are eliciting toxic effects on test organisms. Study specific decision rules for the initial phase of this work are as follows:

"If larval oyster survival in toxicity tests performed with Discovery Bay water is lower than survival in reference area or control water, then the observed mortality is associated with elevated concentrations of one or more substances in Discovery Bay water"

"If one or more metal concentrations in oyster shells from Port Discovery Seafarms are higher than their concentration in oyster shells from a reference or control area, then oysters may have been exposed to elevated metal concentrations during some stage of their life, because oysters are known to detoxify elevated metal concentrations by incorporating the excess metal in their shells, where they are not in contact with organs or soft tissues of the oyster"

6. Specify performance or acceptance criteria

6.1. ***Statistical hypothesis testing.*** Decision-making problems generally are addressed by performing statistical hypothesis tests on the collected data. Decisions are made on whether the data provide sufficient evidence to allow a baseline condition ("null hypothesis", e.g. Surface water pH does not elicit significant effects on larval oyster development and survival) to be rejected in favor of a specified alternative condition ("alternative hypothesis", e.g. Surface water pH reduces the development and survival of larval oysters). For toxicity testing, statistical testing is performed to identify significant adverse effects relative to control performance. The allowable changes in controls are specified in the toxicity test protocol test acceptability criteria. Chemical measurements are usually evaluated as exceedance of the appropriate environmental quality guideline, such as a water quality criterion. Statistical comparison of contaminant concentrations in Discovery Bay water relative to their concentrations in reference area or control samples will commence with tests for normality and homogeneity of variances. If contaminant concentrations pass normality and homogeneity tests, parametric statistical methods (t-test or analysis of variance) will be used to determine any differences in contaminant concentrations between Discovery Bay samples and reference or control samples. If data are not normally distributed, non-parametric statistical methods (Wilcoxon rank sum or Kruskal-Wallis test) will be used to determine any differences in contaminant concentrations between Discovery Bay samples and reference or control samples.

7. Develop the detailed plan for obtaining data

See Appendix A.

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Appendix B – Field Procedures

Appendix C – Site-Specific Data Management Plan

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY REGION 10 1200 Sixth Avenue, Suite 900 Seattle, Washington 98101	**Site-Specific Data Management Plan**			
	Project Name:	Click here to enter text.	**TDD Number/Site ID:**	
	Author:		**Sampling Company:**	
	Date Initiated:	Click here to enter a date.	**Last Updated:**	Click here to enter a date.
	QAPP(s) covering sampling at the site, Approval date:			

This data management plan (DMP) is intended to provide guidance for _____. The Region 10 Data Management Plan acts as a reference guide for this project. All site specific data requirements are considered a sub-set of that region wide DMP. The comprehensive field data deliverable will be a Scribe project managed by the ____ Field team, this project will be published to Scribe.NET daily when samples are shipped or data has been imported into the project. At the conclusion of the project, the .bac file will be delivered to the R10 RSCC and EPA project manager.

Data Processing: The following table outlines the specific requirements for various data types being collected during the project.

	Data Input	Data Stream	Data Provider	Target Database	Site Specific Data Elements	Site Specific Verification	Site Specific SOP
1							
2							
3							
4							
5							

	Reporting Task	Data Inputs	Data Transformation SOP	Deliverable Format(s)	Frequency
1					
2					

Attachments:

QAPP
Sample Alteration Form

Project Name and Number: _____
Sample Matrix: _____
Measurement Parameter: _____

Standard Procedure for Field Collection & Laboratory Analysis (cite reference):
_____
_____

Reason for Change in Field Procedure or Analysis Variation:
_____
_____

Variation from Field or Analytical Procedure:
_____
_____

Special Equipment, Materials or Personnel Required:
_____
_____
_____
_____

Initiators Name: _____ Date: _____

Inspector: _____ Date: _____

Quality Staff: _____ Date: _____

QAPP
Corrective Action Form

Project Name and Number: _____

Sample Dates Involved: _____

Measurement Parameter: _____

Acceptable Data Range: _____

Problem Areas Requiring Corrective Action: _____
_____
_____
_____

Measures Required to Correct Problem(s): _____
_____
_____
_____

Means of Detecting Problems and Verifying Correction: _____
_____
_____
_____

Initiators Name: _____ Date: _____

Inspector: _____ Date: _____

Quality Staff: _____ Date: _____

Appendix D – Health and Safety Plan

Appendix E – Larval-Juvenile Bivalve Toxicity Test Protocol for Pacific Oyster

LARVAL-JUVENILE BIVALVE TOXICITY TEST FOR PACIFIC OYSTER (*CRASSOSTREA GIGAS*): STEP-BY-STEP SUMMARY

INTRODUCTION

The published standard toxicity tests with Pacific oyster (*Crassostrea gigas*) are performed starting with larvae aged between 1 – 4 hours post egg fertilization, and measure the proportion of normal vs. abnormal D-shaped veliger larvae after a 24 hour exposure to test solutions. EPA is unaware of any toxicity test protocol with a bivalve species that tests contaminant toxicity to free swimming larval oysters as they begin to settle onto a substrate. The toxicity test protocol described here is intended to evaluate contaminant effects on free swimming larval oysters several days prior to and as they begin to settle onto a solid substrate. In Pacific oysters, the cessation of the larval planktonic swimming life stage is followed by a crawling behavior where larvae search for a suitable substrate. Ultimately, if successful, larvae cement themselves in place on a solid substrate. This cementation is termed settlement. Prior to cementation, settlement is reversible. The morphogenetic transition from the larval to the juvenile morphology, which normally commences with cementation, is termed metamorphosis and is irreversible. Once cementation has occurred, juvenile oysters are also referred to as spat.

The test protocol described below is based on what was learned during an initial larval oyster toxicity test performed at the EPA Manchester Environmental Laboratory, Port Orchard, WA using only laboratory control water from the NOAA Manchester Research Station laboratory in Port Orchard, WA. This control water is withdrawn from Little Clam Bay, then filtered and sterilized prior to use as a control. EPA identifies reference stations as field locations as representative as possible of what conditions at a test site would be if the test site were substantially free of contaminants. Little Clam Bay does not currently support commercial shellfish growers as does Discovery Bay, and thus is not a representative reference area for Discovery Bay. For this work, the mouth of Discovery Bay in the vicinity of Snow Creek Seafarms, where oyster growth and development is currently occurring without the toxicity observed at Port Discovery Seafarms has been identified as an acceptable reference site.

PREPARATION OF TEST SOLUTIONS

A. The test concentrations and appropriate dilution water will be based on the contaminant(s) to be evaluated. For these initial tests, only ambient Discovery Bay water, process water from Port Discovery Seafarms, reference area water and laboratory control water will be tested without modification.

B. Prepare toxicity test solutions by diluting well mixed unfiltered site water using volumetric flasks and pipettes. Since the purpose of this initial test is to measure toxicity of ambient Discovery Bay surface water, the use of hypersaline brine where necessary to maintain all test solutions at 30 ± 2‰ is unnecessary. Include brine controls in any future tests that use hypersaline brine to adjust salinity to 30 ± 2‰.

C.

D. Prepare a series copper (or zinc) reference toxicant concentrations.

E. Sample study water and reference toxicant solutions for physical/chemical analysis. Measure salinity, pH, water temperature and dissolved oxygen from each test concentration. A minimum

of 100 mL of control, reference or test water in glass beakers is needed in order to perform the daily chemical monitoring.
F. Randomize numbers for test chambers and record the chamber numbers with their respective test concentrations on a randomization data sheet. Store the data sheet safely until after the test samples have been analyzed. NOTE: Toxicity test is invalid if the randomization data sheet is lost, as study results cannot be calculated without it.
G. Place test chambers in a temperature controlled laboratory room, water bath or environmental chamber set to maintain water at 20° C and allow temperature to equilibrate.
H. Measure the temperature daily in one random replicate (or separate chamber) of each test concentration. Monitor the temperature of the water bath or environmental chamber continuously.
I. At the end of the test, measure salinity, water temperature, pH, and dissolved oxygen concentration from each test concentration.

PREPARATION AND ANALYSIS OF EXPOSURE CHAMBERS
A. The test concentrations and appropriate dilution water will be based on the contaminant(s) to be evaluated. For these initial tests, only ambient Discovery Bay water, process water from Port Discovery Seafarms, reference area water and laboratory control water will be tested. Ambient waters will be tested without modification, unless it is necessary to pre-treat exposure water to match conditions used to generate laboratory control water or Port Discovery Seafarms process water (e.g. salinity adjustment, sterilization, filtration, etc.)
B. Prepare test solutions by using well mixed unfiltered or pre-treated site water, as necessary using volumetric pipettes. Use hypersaline brine where necessary to maintain all test solutions at 30 ± 2‰ if necessary based on salinity of collected water. Include brine controls in any tests that use brine.
C. Sample water samples for physical/chemical analysis. Measure and record salinity, pH, water temperature and dissolved oxygen from each test concentration. So as not to disturb test animals, this should be done in beakers without animals, but which are otherwise handled and treated the same as beakers with animals.
D. Test chambers are defined as the smallest physical units between which there are no water connections. Chambers should be covered to keep out extraneous contaminants and bacteria and to minimize evaporation of test solution and material. Tests are conducted in frosted white polystyrene chambers that are 50 mL in capacity. Test chambers contain 40 mL test water/chamber.
E. For each exposure concentration (or test water in this study), a minimum of eight test chambers per test water are prepared. More test chambers per test water may be prepared if desired.
F. Randomize numbers for test chambers and record the chamber numbers with their respective source of test water (or beakers to be used solely for water quality monitoring during the toxicity test) on both the test chambers themselves and a randomization data sheet. Store the data sheet safely until after the test samples have been analyzed.
G. Place test chambers in a temperature controlled laboratory room, water bath or environmental chamber set to 20° C and allow temperature to equilibrate.
H. Measure the test solution temperature daily in a randomly located blank test chamber. Monitor the temperature of the water bath or environmental chamber continuously if possible.

PREPARATION AND ANALYSIS OF TEST ORGANISMS FOR TOXICITY TEST

A. Obtain test organisms and hold as necessary prior to use in toxicity testing. Pacific oyster larvae will be obtained from a commercial supplier, Coast Seafoods in Quilcene, WA. Eyed larvae, which are competent to metamorphose will be shipped damp and cool to the testing laboratory. Although larvae may be maintained in the damp and cool state for several days, it is desirable to use larvae in toxicity testing as quickly as possible upon receipt. Prior to use in toxicity testing, larvae will be placed in filtered natural sea water (30 ± 2‰ salinity) at 20° C in one liter glass containers or plastic bottles (a maintenance container) and maintained on an algal diet of *Isochrysis galbana* (80,000 cells/mL). All experiments will be conducted between 1 and 7 days after receipt of larvae.

B. On day of test, remove larvae from the maintenance container using a precision volumetric pipette to an intermediate 12 mL frosted white polystyrene cup containing filtered natural sea water (30 ± 2‰ salinity) at 20° C. for laboratory controls, or into water temperature and salinity adjusted (if necessary) Port Discovery Seafarms or Snow Creek Oysters surface water Adjust the number of larvae in this larval stock to 10 or more larvae per intermediate container. The purposes of this intermediate container are to facilitate accurate counting of the number of larvae exposed to test waters, and to minimize handling stress on the test organisms. The eyed larvae used at test initiation are shown in Figure 1.

C. Maintain an even distribution of larvae throughout the solution in the larval stock suspension maintenance container by constant gentle stirring of the larvae using a glass rod.

D. Introduce organisms to the eight test chambers per replicate (10 larvae in 3.0 mL of stock) using a 3.0 mL volumetric pipette, which have already been filled with control, reference site or test site water as appropriate. The concentration of larvae in the test solutions should not exceed one larvae per mL of test solution, although concentrations up to 100 larvae/mL do not impair normal development of Pacific oyster larvae. Track how many milliliters of intermediate container water are needed to transfer 10 oyster larvae to each replicate chamber. Add control, reference or test water to the 50 mL polystyrene cups as needed to bring the total water volume to 40 mL. This will result in an initial larval density of one larvae / 4 mL.

E. Toxicity test initiation time occurs when larvae have been added to all test exposure chambers, and the separate set of test chambers without animals to be used for water chemistry analyses have been prepared. Each test, control and reference sample will have a minimum of two replicate sets of eight test chambers per sample.

F. Larval oysters must be fed during the toxicity test in order to maintain acceptable levels of survival. Immediately after test initiation, add *Isochrysis galbana* cells to the exposure chambers so that a concentration of 80,000 ± 20,000 cells/mL of exposure water is attained. Based on previous experience with this algal species from a commercial algal paste, and starting with a stock algal concentration of approximately 18,000,000 cells/mL, 0.05 mL (i.e. one drop) of the stock algal culture contains 900,000 algal cells. This one drop would yield 90,000 cells/mL if added to 10 mL of exposure water. So in chambers with 40 mL exposure water, four drops of the algal stock solution should be added daily to each exposure chamber to maintain 90,000 cells/mL in the exposure water in which oyster larvae are placed during the toxicity test. This volume may need to be adjusted based on the starting count of algal cells/mL of algal paste.

G. Monitor the condition of the animals in all test chambers daily. Provide an additional 80,000 ± 20,000 cells/mL of *Isochrysis galbana* to each exposure chamber 24 hours after test initiation. Also, measure water chemistry data for salinity, pH, water temperature and dissolved oxygen daily.

H. Near the end of the 48-hour exposure period examine several of the controls to determine if development has reached the larval settlement stage. If yes, terminate the test at 48 hours; if no, the test may be continued for up to an additional 24 hours (maximum total exposure of 72 hours) if required to determine if larval metamorphosis has started to successfully occur. A photograph of a larvae that has or is ready to set with its foot extended is shown in Figure 2.
I. Count the number of live and dead larvae and juveniles (i.e. settled and attached oysters are called juveniles or spat in this procedure) under a dissecting microscope, and record the number of each in each test chamber.
J. Terminate the test by addition of hot water to each test chamber containing animals. This thermal shock will kill surviving larvae, and will prevent any live test larvae from being inadvertently discharged to receiving waters.
K. Perform final water chemistry analyses for salinity, pH, water temperature and dissolved oxygen.
L. Determine if larval survival and the conditions under which the toxicity test was performed meets the test acceptability criteria presented in Table 1. If so, statistically analyze the data to determine if survival and/or setting rate significantly differ between test samples and the control and reference samples. Statistical testing procedures are described in the data quality objectives document for this work.
M. If run, include standard reference toxicant point estimate values in the standard quality control charts.

Table 1. Summary of test conditions and test acceptability criteria for *Crassostrea gigas*, larval-juvenile development test.

1. Test type:	Static non-renewal
2. Salinity:	30 ± 2‰
3. Temperature:	20 ± 1° C
4. Light quality:	Ambient laboratory light
5. Light intensity:	10-20 µE/m^2/s (Ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	50 mL
8. Test solution volume:	40 mL
9. No. larvae per chamber:	10
10. No. replicate chambers per concentration:	8
11. No. rows of replicate chambers per concentration	Minimum of 2
12. Dilution water:	Uncontaminated 1-µm-filtered natural seawater or hypersaline brine prepared from natural seawater
13. Test concentrations:	Effluents: Minimum of 5 and a control Receiving waters: 100% receiving water and a control
14. Dilution factor:	Effluents: x0.5 Receiving waters: None or x0.5
15. Test duration:	48 hours (or until larval settlement begins, up to 72 hours maximum exposure)
16. Feeding regime:	*Isochrysis galbana*, added daily to achieve nominal 80,000 algal cells / mL test solution
17. Endpoint:	Survival and normal larval settlement
18. Test acceptability criteria:	Control and reference sample survival must be ≥70% for oyster larvae; with ≥50% survival of settled larvae in control and reference samples; and must achieve a %MSD of <25%
19. Sampling requirements:	One sample collected at test initiation, and preferably used within 24 h of the time it is removed from the sampling device
20. Sample volume required:	2 L per test

Figure 1. Photograph of Representative Eyed Pacific Oyster Larvae Used at Start of Toxicity Test.

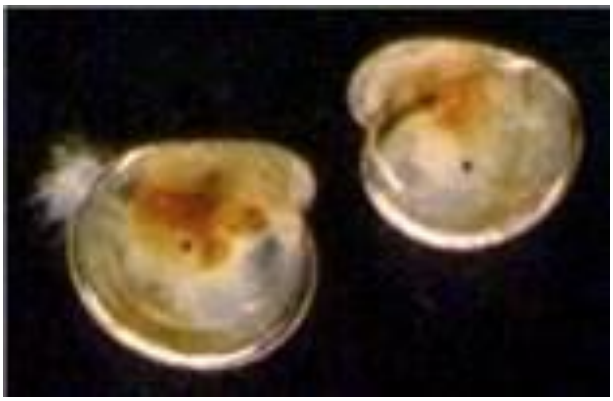

Figure 2. Photograph of Representative Setting Pacific Oyster Larvae with Transparent Foot Extended.